

Long-term adaptation of pancreatic response by dogs to dietary fats of different degrees of saturation: olive and sunflower oil

By M. C. BALLESTA, M. MAÑAS, F. J. MATAIX, E. MARTÍNEZ-VICTORIA*
AND I. SEIQUER

Instituto de Nutrición y Tecnología de Alimentos, Departamento de Fisiología, Facultad de Farmacia, Universidad de Granada, Granada, Spain

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Mongrel dogs were fed, from weaning to 9 months of age, on one of two diets that differed only in the type of fat content (virgin olive oil or sunflower oil) to compare the composition of exocrine pancreatic secretion in the basal period and in response to food. In resting pancreatic flow, electrolytes and the specific activities of amylase, lipase and chymotrypsin were similar in both experimental groups. However, lipase and amylase outputs, and amylase and protein concentrations were significantly higher in the group fed on the diet rich in sunflower oil. Food intake was not followed by any change in flow-rate or electrolyte or protein content in the group given the diet rich in olive oil. Amylase activity and output were also lower in this group, as was lipase output, whereas activity and specific activity of chymotrypsin were lower in dogs fed on the diet containing sunflower oil. The differences traceable to the composition of the two types of dietary fat supplied may be related to the balance between factors that stimulate and inhibit pancreatic secretion.

Dietary fat: Pancreatic secretion: Dog

The adaptation of pancreatic enzymes to the type of food available is one of the physiological advantages that allow animals to use nutrients and energy for metabolism as efficiently as possible. A number of studies have analysed the adaptation of pancreatic enzymes to the presence of different substrates in the diet. Rising levels of dietary fat lead to parallel increases in lipase (EC 3.1.1.3) activity in rat pancreas homogenates (Bucko *et al.* 1969; Robberecht *et al.* 1971; Deschodt-Lanckman *et al.* 1971; Snook, 1971; Sabb *et al.* 1986; Bazin & Lavan, 1979; Wicker & Puigsever, 1987). The degree of unsaturation appears to have no effect on pancreatic levels of lipase (Saraux *et al.* 1982), whereas the length of the fatty acid chain has been shown to influence lipase levels in this organ (Saraux *et al.* 1982).

In the rat, the amount of dietary fat affects amylase (EC 3.2.1.1) activity and protease content in the exocrine pancreas. Changes in amylase are the inverse of those in lipase: amylase activity declines as the amount of dietary fat increases (Deschodt-Lanckman *et al.* 1971; Snook, 1971; Saab *et al.* 1986; Wicker & Puigsever, 1987). The extent of this decrease is influenced by the length and the degree of unsaturation of dietary fatty acids (Saraux *et al.* 1982). Trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) are modified in the same direction as lipase when the amount of dietary fat rises (Snook, 1971; Wicker & Puigsever, 1987).

Although most studies on the adaptation of pancreatic enzyme levels have been performed in the rat, other species used to date include the dog (Behrman & Kare, 1969), the pig (Corring & Saucier, 1972; Low, 1982; Partridge *et al.* 1982; Simoes-Nunes, 1985), and man (Ekelund & Johanson, 1980; Emde *et al.* 1985). While the overall patterns of

* For reprints.

adaptation seen in these species are partially comparable to those in the rat, the differences become more significant between this animal and man.

The presence of fat, especially its digestive products the monoglycerols and fatty acids, in different segments of the intestine is known to affect the volume as well as the bicarbonate and enzyme content of exocrine pancreatic secretion (Meyer & Jones, 1974; Yamagishi & Debas, 1980; Faichney *et al.* 1981; Malagelada *et al.* 1985). To date, however, no studies have dealt with the long-term pancreatic adaptation to high levels of specific nutrients in the diet. Excessive amounts of dietary fat can affect not only hydrolase levels in tissue and pancreatic juice but also the overall pattern of pancreatic response to the presence of food in the digestive tract.

The present article describes the long-term adaptation of exocrine pancreatic secretion in the dog to high levels of two types of dietary fat, which differ in fatty acid unsaturation: sunflower oil, rich in polyunsaturated fatty acids, and virgin olive oil, a fat source of high monounsaturated fatty acid content. The effects of these two diets were analysed in terms of (1) pancreatic response to food and (2) enzyme content in the pancreatic juice secreted.

MATERIALS AND METHODS

Animals

The subjects were mongrel dogs of both sexes weighing 15–25 kg. The animals were weaned (at 15–20 d of age) and randomly assigned to one of the two experimental groups. Subjects in group S (four animals) and group O (four animals) were fed on diets in which the source of fat was sunflower oil and olive oil respectively. During the 8-month adaptation period, all dogs were housed in individual cages with free access to water. Feeding took place once daily between 09.30 and 12.00 hours to adapt the animals to the experimental regimen and avoid conditioning to a specific feeding time.

Diets

The diets used were prepared from commercial dog chow (Extra[®], Gabrina Purina) made up specially for the present study, omitting fat. Sunflower oil (Koipesol[®]; Koipe S.A., Jaén, Spain) was added to the chow given to group S, and virgin olive oil (Patrimonio Comunal Olivarero, Madrid, Spain) was added to the chow given to group O animals.

There were no marked differences between the plant sterol content or tocopherol content in the two dietary fats, the concentration of which was very small compared with the saponifiable fraction.

Antioxidant levels or peroxide values were not measured because the diets were prepared every day, and the correct amount of 'fresh' oil (olive or sunflower) added to avoid peroxidation. The two oils were stored in 1 litre closed bottles in the dark for short periods (no longer than 1 week) before use.

Table 1 summarizes the composition of the two diets, which aside from their different fat sources were isoenergetic and isonitrogenous and thus differed only in fatty acid composition. Table 2 shows the percentage content of each fatty acid in both diets.

Surgical preparation

After a 20 h fast with free access to water the animals were anaesthetized with a 30 mg/kg body-weight dose of sodium thiopental (Tiobarbital[®]; Miró S.A., Jaén, Spain). Intubation was performed under direct laryngoscopic vision using a Rush-type pressure-cap tube. During surgery the animals were connected to a Boyle-type open respiratory system (Medishield; B.O.C., London) using a mixture of oxygen and nitrous oxide bubbled through a Halothane solution (Ethrane[®]; Abbot Laboratories S.A., Madrid, Spain).

Table 1. *Composition of the experimental diets*

| | Sunflower oil diet | | | Olive oil diet | | |
|--------------|--------------------|------|------------|----------------|-----|------------|
| | g/kg | | Energy (%) | g/kg | | Energy (%) |
| | Mean | SE | | Mean | SE | |
| Protein | 218 | 4.5 | 19.4 | 222 | 3.9 | 19.4 |
| Fat | 159 | 9.0 | 31.9 | 174 | 4.6 | 34.4 |
| Carbohydrate | 546 | 11.1 | 48.7 | 527 | 4.9 | 46.2 |
| Ash | 77 | 2.0 | — | 77 | 1.8 | — |

The dog chow (Extra® Gabrina Purina), which was the basal diet for the above experimental diets, contained (g/kg): dry matter 880, crude protein 210, fat 0, fibre 35, ash 80, sodium chloride 12, calcium 15, phosphorus 9, choline 2 and (mg/kg): iodine 1.3, magnesium 80.0, copper 30.0, iron 400.0, zinc 140.0, cobalt 1.0, retinol equivalent 6.0, vitamin E 13.4, thiamin 10.0, riboflavin 8.0, pyridoxine 10.0, cyanocobalamin equivalent 0.3, menadione 1.0, cholecalciferol equivalent 0.05, nicotinic acid 80.0, folic acid 5.0, pantothenic acid 25.0.

Table 2. *Fatty acid composition of experimental diets (% total fatty acid content)*

| Fatty acid | Sunflower oil diet | Olive oil diet |
|------------------------------|--------------------|----------------|
| Palmitic (16:0) | 9.1 | 11.7 |
| Stearic (18:0) | 3.5 | 2.4 |
| Oleic (18:0 <i>n</i> -9) | 25.5 | 60.9 |
| Linoleic (18:2 <i>n</i> -6) | 56.3 | 15.3 |
| Linolenic (18:3 <i>n</i> -3) | 2.3 | 0.8 |

After medial laparotomy, the main pancreatic duct was cannulated according to the technique of White & Murat (1967), using a silastic cannula with a rigid PVC tip. Near the drainage of the main pancreatic duct, a 'T' cannula was implanted in the duodenum and fixed to the intestinal wall with purse-string sutures. Both cannulas were exteriorized through the right abdominal wall. The experiments began 1 week after surgery.

Time-course of the experiments

The animals were deprived of food but not water for 20 h before starting the experiments. After collecting two 60 min samples of pancreatic juice, the dogs were fed and pancreatic secretions were sampled hourly for the next 5 h. All samples were collected into PVC bags. Flow-rates were determined gravimetrically with density considered equal to 1 (Hoffman, 1963). A portion was taken from all samples for the immediate determination of bicarbonates, and a portion of each sample was frozen at -20° for subsequent analysis. The rest was recirculated into the duodenum to avoid possible interferences.

Together with pancreatic juice, the duodenal contents were sampled at the same time intervals to determine pH (Crisson® pH meter, model MicropH 2001; Crisson Instruments S.A., Barcelona, Spain).

Analytical methods

Bicarbonates were determined in 0.5 ml pancreatic juice to which 5.0 ml 0.01 M-hydrochloric acid was added, followed by heating to the boiling point and cooling. The acid residue was neutralized with 0.01 M-sodium hydroxide, and the results were expressed in mmol/l (Debas & Yamagishi, 1978).

Amylase activity was measured by hydrolysis of the starch substrate and determination

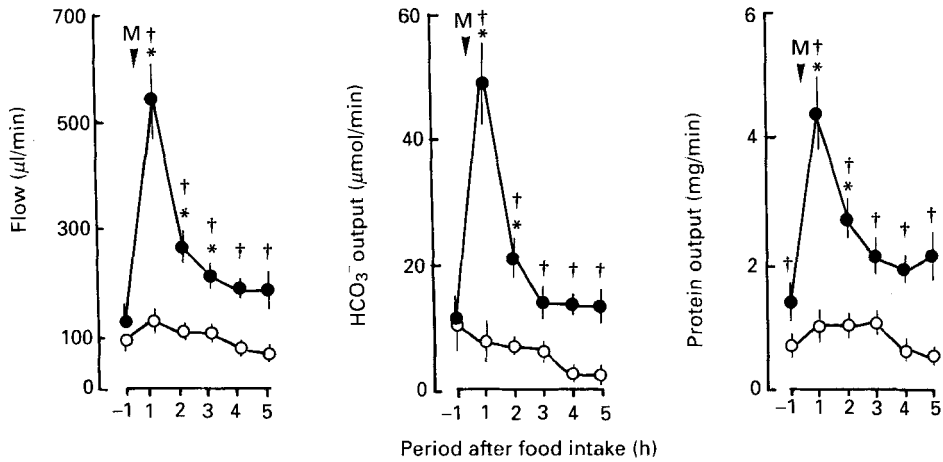


Fig. 1. Volume of pancreatic juice flow, and bicarbonate and protein output under resting conditions (time -1) and after food intake (M) in dogs fed on diets containing sunflower oil (●) and olive oil (○). Values are means, with their standard errors represented by vertical bars for three experiments/dog. Mean values for the two dietary groups were significantly different from those under resting conditions: * $P < 0.05$. Mean values for the two dietary groups were significantly different: † $P < 0.05$.

of the amount of maltose released, in accordance with the technique described by Noelting & Bernfield (1948), as modified by Hickson (1970). The results were expressed in units of activity as defined by Hickson (1970).

Lipolytic activity was determined using the technique of Negrel *et al.* (1976), by titration at a constant pH (8.5) with tributyrine as the substrate. The unit of lipase activity was defined as that amount of lipase able to release 1 µmol acid/min at 27°.

To determine chymotrypsin activity, titration was performed at a constant pH (7.9) with acetyltyrosine ethyl ester (ATEE) as the substrate. Chymotrypsin was previously activated with trypsin. Activity was expressed in µmol ATEE hydrolysed/min at 27° (Rebound *et al.* 1962).

Protein was determined as described by Bradford (1976).

Statistical treatment

For comparisons of the means within and between experimental groups, Mann-Whitney's non-parametric *U* test and Student's *t* test were used. The differences were considered significant when $P \leq 0.05$.

RESULTS

Food intake

There were no differences in food intake between the groups. All the animals ate the same amount of food (500 g/d) and they had the same energy intake during the experimental period.

Resting secretion

The pancreatic flow-rate was similar in both experimental groups, slightly but not significantly higher in dogs fed on the diet containing sunflower oil (Fig. 1). Likewise, bicarbonate levels were not significantly different (Fig. 1). However, protein content was higher in juice secreted by group S animals ($P < 0.05$) (Fig. 1), and amylase activity as well as the output of this enzyme were also significantly greater in this group (Fig. 2). Lipase secretion, but not activity, was significantly higher in animals given sunflower oil (Fig. 2).

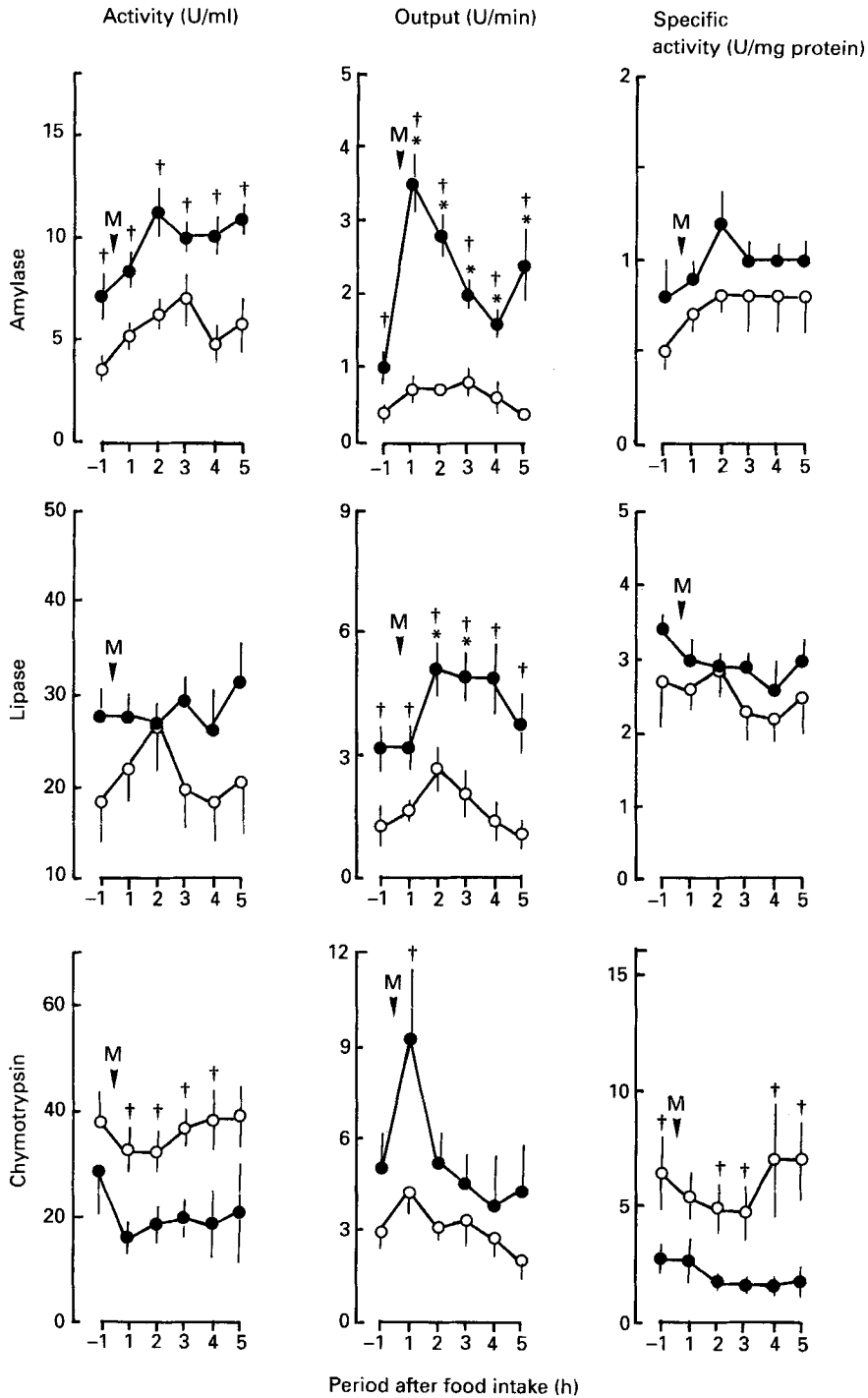


Fig. 2. Activity, output and specific activity of amylase (*EC* 3.2.1.1), lipase (*EC* 3.1.1.3) and chymotrypsin (*EC* 3.4.21.1) under resting conditions (time -1) and after food intake (M) in dogs fed on sunflower seed oil (●) and olive oil (○). Values are means, with their standard errors represented by vertical bars for three experiments/dog. Mean values for the two dietary groups were significantly different: † $P < 0.05$. Mean values for the two dietary groups were significantly different from those under resting conditions: * $P < 0.05$.

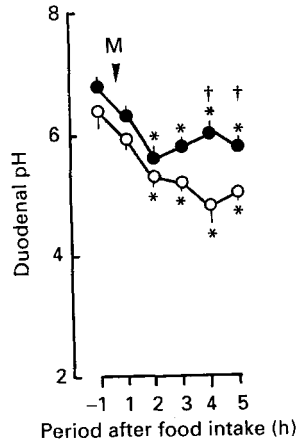


Fig. 3. Duodenal pH values under resting conditions (time -1) and after food intake (M) in dogs fed on sunflower seed oil (●) and olive oil (○). Values are means, with their standard errors represented by vertical bars for three experiments/dog. Mean values for the two dietary groups were significantly different: † $P < 0.05$. Mean values for the two dietary groups were significantly different from those under resting conditions: * $P < 0.05$.

Despite the slightly higher value for chymotrypsin activity in pancreatic juices from group O, the difference was not statistically significant (Fig. 2). The specific activities of amylase and lipase were similar in both groups, while chymotrypsin specific activity was significantly higher in dogs fed on virgin olive oil (Fig. 2).

Response to food

The two groups responded differently to the presence of food in the digestive tract. In group S, there was a significant increase in the volume of pancreatic juice secretion during the first 3 h after food intake, while the flow of pancreatic secretion showed no change throughout the entire postprandial period in group O animals (Fig. 1). Overall, flow was greater in group S during the entire period of study.

In animals given the diet high in polyunsaturated fatty acids, food intake led to a marked rise in bicarbonate levels during the first 2 h after eating. In group O, however, no significant change was recorded throughout the entire postprandial period. Bicarbonate production was consistently higher in group S dogs (Fig. 1). A very similar pattern was shown by protein output (Fig. 1).

Amylase activity in the pancreatic juice showed no significant change after food intake in either group. The activity of this enzyme was nevertheless consistently higher in group S (Fig. 2). Amylase output rose significantly after feeding and remained elevated during the following 5 h, in contrast with the lack of change in group O during this period. Specific activity of amylase behaved similarly in both groups, with no significant changes in response to food. No significant difference between the two groups was found in postprandial specific amylase activity.

Lipase activity and specific activity showed no significant change in response to food in either of the experimental groups. Neither of the two variables differed significantly when the two groups were compared. Lipase output, however, rose significantly in group S at 2 and 3 h postprandial. In group O lipase output increased in response to food intake, but the changes were not significant. Lipase production was significantly lower in group O than in group S throughout the experimental period (Fig. 2).

Finally, chymotrypsin activity and specific activity did not change significantly in

response to food in either group. Olive oil, however, led to significantly higher values for both variables than sunflower seed oil. At 1 h after feeding, chymotrypsin production had risen in group S to be significantly higher than in group O, whereas no change was seen in group O. No further significant differences between the groups were recorded during the remainder of the experimental period (Fig. 2).

Duodenal pH

Resting pH values of the duodenal contents were similar in both groups (6.4 (SE 0.25) with olive oil; 6.8 (SE 0.22) with sunflower seed oil). The presence of food led to a decline in pH which became significant from the second hour postprandially in both groups. Significant differences between the groups were not apparent until 4 and 5 h after food intake, when duodenal pH was lower in group O than in group S (Fig. 3).

DISCUSSION

Resting conditions

Under resting conditions and after the period of adaptation to the diet, mean pancreatic juice flow was similar to that recorded previously by Madrid *et al.* (1985) and Huertas *et al.* (1985) in dogs under identical experimental conditions, fed on a commercial dog chow ranging in fat content from 30 to 70 g/kg. This suggests that the volume of pancreatic juice secreted in the absence of stimuli is unaffected by the amount or degree of unsaturation of the dietary fatty acids. The production of bicarbonate under the present experimental conditions was significantly higher in comparison with the values obtained with low-fat diets in the two previously mentioned studies. No study to date has described the adaptation of bicarbonate secretion to different amounts of dietary fat, although high-fat diets as well as the intralumen administration of fatty acids have been shown to stimulate both the hydroelectrolytic and the organic component of the pancreatic juice secreted (Meyer & Jones, 1974). Raising the amount of dietary fat also modifies plasma levels of secretin and cholecystokinin (CCK) (Modlin *et al.* 1979; Yamagishi & Debas, 1980). We believe the rise in bicarbonate levels in pancreatic secretions may be related to changes in the level of these hormones in response to the increase in dietary fat.

The differences seen in the production of protein between groups S and O in the present study suggest that the degree of fatty acid unsaturation influences plasma protein levels. Several earlier studies have shown (Fink *et al.* 1983a; Hopman *et al.* 1985; Konturek *et al.* 1986) that oleic acid (the major fatty acid in the diet given to group O animals) is the most potent releaser of CCK, itself a potent stimulant of the protein fraction of exocrine pancreatic secretion. However, oleic acid is also known to be a potent releaser of other gastrointestinal peptides able to inhibit exocrine pancreatic secretion, including peptide YY (PYY) (Pappas *et al.* 1985, 1986; Aponte *et al.* 1985) and PP (Fink *et al.* 1983b). In dogs, the release of PYY by oleic acid has been shown to inhibit pancreatic secretion of proteins stimulated by submaximal doses of CCK (Pappas *et al.* 1985). This leads us to think that the reduced production of pancreatic proteins in the animals fed on olive oil may have resulted from the balance between the stimulating (CCK) and inhibiting (PYY and PP) effects of oleic acid (the major fatty acid in olive oil) on the protein fraction of pancreatic secretion.

The values for amylase activity and production published by Madrid *et al.* (1985) for dogs given a diet containing 50% less fat than in our study were markedly higher than our values for both groups. The differences may be due to the process of adaptation to high-fat diets, as manifested in the rat pancreas by increases in lipase activity accompanied by

a reciprocal decline in amylase activity (Deschodt-Lanckman *et al.* 1971; Snook, 1971; Saraux *et al.* 1982). The lower values in dogs given olive oil in the present study may reflect, as pointed out by Deschodt-Lanckman *et al.* (1971), the greater effectiveness of olive oil in inhibiting amylase, as compared with other sources of dietary fat such as tricaprylin and maize oil.

The activity and specific activity of lipase were apparently unaffected by the type of dietary fat provided and, thus, appear to change only in response to rising quantities of lipids (Bazin & Lavan, 1979; Saraux *et al.* 1982; Sabb *et al.* 1986).

Response to food

Qualitative and quantitative differences between group S and group O were seen in pancreatic response to the two sources of dietary fat studied. Considering the values of duodenal pH and the composition of the diets, the secretory response to food would be expected to show patterns similar to those described by Madrid *et al.* (1985) and Huertas *et al.* (1985), who fed their animals with standard dog chow. The response may have been somewhat delayed, while the animals were fed with high-fat diets, as a result of delayed gastric emptying. The different responses to food in experimental groups S and O may be explainable in terms of the balance between those factors that stimulate and inhibit postprandial pancreatic secretion. The lack of response to food, as determined by the amount of bicarbonate and protein in pancreatic juice, may be traceable to the effect of oleic acid (in animals given olive oil) on the various factors involved in pancreatic response to food, as described previously for the resting condition. This monounsaturated fatty acid has been shown to be a potent stimulant of CCK (Hopman *et al.* 1985; Konturek *et al.* 1986) and secretin release (Kim *et al.* 1980; Fink *et al.* 1983*a*), both of which are potent stimulants of pancreatic secretion. Oleic acid is also the most effective releaser of gastrointestinal peptides that inhibit both basal exocrine pancreatic secretion and secretion stimulated by submaximal doses of secretin and CCK, including PYY (Aponte *et al.* 1985; Pappas *et al.* 1986) and PP (Fink *et al.* 1983*b*). Thus, the factors that favour and impede pancreatic secretion would reach equilibrium, leading in essence to a null change in pancreatic response (in terms of bicarbonate and protein levels) to the presence of food in the digestive tract.

The enzyme content in pancreatic juice secreted in response to food showed the same trends as were recorded under resting conditions. Amylase activity and production were lower in group O, probably owing to the potent inhibitory effect of olive oil on amylase in comparison with other lipids such as tricaprylin and maize oil (Deschodt-Lanckman *et al.* 1971). Lipase production, but not activity, was affected by the degree of unsaturation of the dietary fat tested (Saraux *et al.* 1982). Chymotrypsin and amylase values showed opposite trends. Chymotrypsin activity and specific activity were higher in group O animals throughout the postprandial period studied. The high oleic acid content of olive oil may explain the behaviour of these proteases, as this fatty acid is a potent releaser of CCK (Konturek *et al.* 1986), a hormone known to stimulate the organic component of pancreatic secretion (Mutt & Jorpes, 1971; Modlin *et al.* 1979; Stubbs & Stabile, 1985), especially proteolytic enzymes including chymotrypsin (Folsch *et al.* 1978).

Taken together, our findings show that exocrine pancreatic secretory activity in response to the presence of food is greater in dogs fed on a diet rich in sunflower seed oil (high polyunsaturated fatty acid content) than in animals given the same diet with virgin olive oil (rich in monounsaturated fatty acids) as the source of fat. In the latter group, postprandial secretory activity hardly changed in comparison with secretion under resting conditions. Parallel experiments have shown not only that there are no significant differences between group O and group S animals in digestive utilization of dietary fat and protein, but that in

some cases the indices of nutritive utilization are actually higher in dogs adapted to a diet containing virgin olive oil (M. Mañas, F. J. Mataix, E. Martínez-Victoria and I. Seiquer, unpublished results). Such adaptation to dietary olive oil is a key advantage in terms of exocrine pancreatic economy, at least in this species.

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